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Invited Minireview

Afferent and efferent immunological pathways of the brain. *Anatomy, Function and Failure*

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ABSTRACT

Immunological privilege appears to be a product of unique lymphatic drainage systems for the brain and receptor-mediated entry of inflammatory cells through the blood–brain barrier. Most organs of the body have well-defined lymphatic vessels that carry extracellular fluid, antigen presenting cells, lymphocytes, neoplastic cells and even bacteria to regional lymph nodes. The brain has no such conventional lymphatics, but has perivascular pathways that drain interstitial fluid (ISF) from brain parenchyma and cerebrospinal fluid (CSF) from the subarachnoid space to cervical lymph nodes. ISF and solutes drain along narrow, ~100 nm-thick basement membranes within the walls of cerebral capillaries and arteries to cervical lymph nodes; this pathway does not allow traffic of lymphocytes or antigen presenting cells from brain to lymph nodes. Although CSF drains into blood through arachnoid villi, CSF also drains from the subarachnoid space through channels in the cribriform plate of the ethmoid bone into nasal lymphatics and thence to cervical lymph nodes. This pathway does allow the traffic of lymphocytes and antigen presenting cells from CSF to cervical lymph nodes. Efferent pathways by which lymphocytes enter the brain are regulated by selected integrins on lymphocytes and selective receptors on vascular endothelial cells. Here we review: (1) the structure and function of afferent lymphatic drainage of ISF and CSF, (2) mechanisms involved in the efferent pathways by which lymphocytes enter the brain and (3) the failure of lymphatic drainage of the brain parenchyma with age and the role of such failure in the pathogenesis of Alzheimer's disease.

1. Introduction

The concept of immunological privilege was introduced in 1921, following the observation that allografts implanted into the brain survived for a longer period than allografts in other tissues (Ridley and Cavanagh, 1969). This concept was reinforced by the discovery of the blood–brain barrier (BBB) and became generally accepted in the first half of the 20th century. We know now that the endothelium has different barrier properties depending on the type of cerebral blood vessel and that immune privilege represents a spectrum of observations that are applied to the extracellular compartment of the brain, rather than a complete isolation of the brain and CSF from the immune system (Galea et al., 2007; Bechmann et al., 2007; Muldoon et al., 2013). Tissue grafts, bacteria, viruses implanted in the brain parenchyma escape systemic immune recognition, although they elicit a robust immune response in the CSF compartment, as reviewed in Galea et al. (2007). In other tissues, antigens are taken up by dendritic cells and presented to T cells that initiate an immune response. Dendritic cells do not reside in the brain parenchyma except for small numbers in the periventricular regions and we know that cells do not migrate out from the normal brain parenchyma (Galea et al., 2007). Cells that express markers characteristic for dendritic cells (CD11b, CD11c) are present only in the CSF compartment and in the inflamed CNS parenchyma (Galea et al., 2007). Although most tissues in the body have well defined lymphatic channels for the drainage of fluid, solutes and cells, the brain has no such conventional lymphatic vessels. Soluble antigens are able to drain from the brain parenchyma towards the cervical lymph nodes along perivascular pathways, but the exact connections between the ISF and CSF compartments are still under investigation. An allograft or bacteria implanted into the brain parenchyma do not trigger a local immune response, but if the graft is implanted in the skin or if the bacteria are inoculated systemically following the implantation into the brain, an aggressive immune response occurs in the brain (Galea et al., 2007). These observations, strengthened by the pres-

Abbreviations: BM, basement membrane (green contains tracer); SMC, smooth muscle cell in the tunica media of the artery; IC, immune complexes derived from the blood and entrapped in basement membranes of the perivascular drainage pathways.

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ence of immunological reactions in the brain in response to virus infections, in multiple sclerosis and in experimental autoimmune encephalomyelitis (EAE), challenge the rigid concepts of immune privilege and blood–brain barrier and suggest that the different compartments of the brain have modified anatomical and functional immunological characteristics, compared to the rest of the body (Laman and Weller, 2013).

There are two major extravascular, extracellular fluids associated with the brain. Interstitial fluid (ISF) is present in brain parenchyma and cerebrospinal fluid (CSF) fills the cerebral ventricles and the subarachnoid spaces surrounding the brain and spinal cord. Although there are no conventional lymphatics in the brain, both ISF and CSF have well defined lymphatic drainage pathways, albeit unconventional, that are largely separate from one another and drain fluid and solutes from the brain to cervical lymph nodes. In this review, we present evidence for distinct anatomical pathways for the lymphatic drainage of ISF and for CSF; we then discuss the anatomical and physiological features of these afferent pathways and their roles in immunological reactions in the brain. We examine the selective nature of the efferent immunological pathway by which lymphocytes enter the brain by receptor-mediated pathways through the vascular endothelium. Finally, we assess how age changes in cerebral blood vessels disrupt lymphatic drainage of amyloidogenic proteins from the brain. Failure of lymphatic drainage appears to be a factor in the accumulation of amyloid-β (Aβ) in the brain and artery walls in Alzheimer's disease (AD); the accumulation of fluid in the white matter (leukoaraiosis) in the ageing brain and the failure of protein elimination in other dementias (Carare et al., 2013).

2. Structure and function of afferent lymphatic drainage of ISF and CSF from the brain

2.1. Lymphatic drainage of interstitial fluid from the brain parenchyma

ISF is present in the extracellular spaces of grey and white matter in the brain and in humans has a total volume of 280 ml. It is derived from the blood and from metabolic activity in the brain and is eliminated by bulk flow at a rate of 0.1–0.3 μl min^{-1} g^{-1} from the rat brain (Abbott, 2004). Injections of radioactive tracers into the basal ganglia of experimental animals resulted in drainage of such tracers to cervical lymph nodes at a speed that is similar to lymphatic drainage in other tissues (Szentistvanyi et al., 1984). Furthermore, such studies have shown that the drainage pathway for ISF to cervical lymph nodes is along the walls of cerebral arteries. More recent injection studies using fluorescent tracers combined with immunocytochemistry and high-resolution confocal microscopy have shown that solutes injected into ISF of the brain are eliminated along basement membranes, 100 nm thick, in the walls of cerebral capillaries and cerebral arteries (Fig. 1A, parts 1 and 2) (Carare et al., 2008). Thus, it appears that the lymphatic drainage of the brain for fluid and solutes is along restricted pathways within the walls of cerebral blood vessels. However this pathway is not large enough for the drainage of particles 0.02 μm in diameter or for antigen presenting cells and lymphocytes from the brain (Carare et al., 2008). The lack of direct trafficking of antigen presenting cells and lymphocytes from the brain parenchyma to cervical lymph nodes may be a major factor in the observed immunological privilege in the brain.

Theoretical models and results from experimental studies suggest that the motive force for perivascular drainage of ISF and solutes is derived from vascular pulsations (Schley et al., 2006). Thus, the contrary wave that follows the pulse wave in cerebral arteries, but in the reverse direction, may drive fluid and solutes out of the brain along the basement membranes within vessel walls. This model has relevance for the apparent failure of perivascular lymphatic drainage with age as arteries stiffen with age and arteriosclerosis (Schley et al., 2006). Recent studies in which solutes clearance has been directly visualised have shown that there is decreased clearance of solutes when vascular perfusion is impaired. These studies emphasise the probable role of vascular pulsations in the clearance of ISF and solutes from the brain (Arbel-Ornath et al., 2013).

2.2. Lymphatic drainage of CSF

CSF is produced by the choroid plexuses in the cerebral ventricles and passes into the subarachnoid spaces surrounding the brain and spinal cord. A proportion of CSF drains into the blood via arachnoid villi in venous sinuses (Fig. 1A, parts 8 and 9), but CSF also drains from the cerebral subarachnoid space to cervical lymph nodes (Fig. parts 4–7) (Wolburg and Paulus, 2010). Although arachnoid villi and granulations are well developed in humans, they are small in other mammals such as the rat and it is estimated that at least 50% of CSF drains to cervical lymph nodes in these animals. Injection of particulate matter such as Indian ink into the CSF in rodents and primates has outlined channels that drain CSF from the subarachnoid space through well-defined channels that accompany branches of olfactory nerves through the cribriform plate of the ethmoid bone in rats (Fig. 1A, parts 4 and 4a),(Zhang and Richards, 1992). The trans-ethmoid channels are continuous with nasal lymphatics and the Indian ink drains to cervical lymph nodes (Fig. 1A, part 5). Similar lymphatic drainage channels have been identified along spinal nerves, providing an alternate drainage pathway for the CSF in hydrocephalus (Voelz et al., 2007). The exact anatomy of the extensions of the subarachnoid spaces along cranial and spinal nerves requires more investigation as these pathways provide an important site for immunological reactions and entrapment of tumour or inflammatory cells (Schmitt et al., 2011).

2.3. Interrelationships between ISF and CSF

Although ISF and CSF are in largely separate compartments and their drainage pathways appear to be separate, the two fluids are interrelated. Injection of tracers into the CSF results in penetration of tracer along periarterial pathways into the brain and diffusion into ISF (Iliff et al., 2012). The entry pathway for CSF into the brain is not the same as the intramural basement membranes in the walls of arteries that represent the lymphatic drainage pathway for ISF (Carare et al., 2008). Although the exact functional relationship between the infusion of CSF into brain tissue and lymphatic drainage of ISF is at the moment unclear, the relationships could change with age and other factors that influence interstitial fluid drainage, like expression of aquaporins that control the transit of water across the astrocyte end-feet (Nedergaard, 2013). Recent data suggest that the pathways of entry of lipophilic and hydrophilic tracers from the CSF into the brain parenchyma are similar and involve penetrating arteries and veins (Rangroo Thrane et al., 2013). Elimination of solutes from brain parenchyma into the CSF may be a pathway that compensates for blockage of interstitial fluid drainage in AD but interstitial fluid drainage does not seem to compensate for blockage of CSF drainage as in hydrocephalus.

2.4. Significance of lymphatic drainage for immunological reactions in the brain

Experimental studies have shown that cervical lymph nodes play a role in immune reactions in the brain. When EAE is induced in rats by the peripheral injection of antigen and Freund's adjuvant into the foot, inflammation with lymphocyte infiltration and